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(54) Detecting a selected species

(57) A selected species (e.g. an antibody or an antigen) is detected by monitoring the effect of a modification of diffusion of a donor and/or an acceptor upon energy transfer from the donor to the acceptor; the modification of diffusion results from interaction with the selected species.

The invention finds application in, for example, immunoassay and immunosensing.

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Detecting a Selected Species

The present invention relates to detecting a selected species (e.g. an antibody or an antigen).

According to one aspect of the present invention there is provided a method for detecting a selected species which comprises monitoring the effect of a modification of diffusion of a donor and/or of an acceptor upon energy transfer from the donor to the acceptor, the modification of diffusion resulting from interaction with the selected species.

By monitoring the effect that modification of diffusion has upon energy transfer between the donor and acceptor it is possible to monitor a change in the diffusion coefficient of the donor and/or acceptor.

The energy transfer between a donor and an acceptor may be monitored, for example, by applying excitation energy (e.g. by means of a xenon flash lamp) and monitoring energy emission of the donor and/or acceptor.

The energy emission of the donor may be observed, for example, as fluorescence energy or as phosphorescence energy; likewise the energy emission of the acceptor may be observed, for example, as fluorescence energy or phosphorescence energy.

In accordance with the present invention the diffusion coefficient of an energy donor and/or of an energy acceptor may be modified by an interaction involving the selected species whereby diffusion enhanced energy transfer from a donor to an acceptor is affected. (In the case of energy transfer from a fluorescence energy donor to a fluorescence energy acceptor, diffusion enhanced fluorescence energy

transfer (DEFET) may be utilised). For example, the selected species may be arranged to interact with the donor and/or with the acceptor so as to reduce the transitional diffusion of the donor and/or of the acceptor during the lifetime of the donor in an excited state whereby the rate of energy transfer from donor to acceptor is reduced. Thus, for example, by monitoring the energy emission signal of the donor and/or acceptor after excitation in the absence and then the presence of a selected species the presence of the selected species may be detected.

In the circumstances described, by way of example, in the immediately foregoing paragraph, the donor energy signal (e.g. the fluorescence energy signal or phosphorescence energy signal) will be relatively low and the acceptor energy signal (e.g. the acceptor fluorescence energy signal as phosphorescence energy signal) will be relatively high in the absence of the selected species due to diffusion enhanced energy transfer from donor to acceptor; on addition of a selected species to be detected, which species interacts so as to reduce the diffusion coefficient of the donor and/or acceptor, the rate of energy transfer from donor to acceptor is reduced and hence the donor signal increases and the acceptor signal decreases. In this way the presence of the selected species may be detected.

In principle any suitable interaction of the selected species which leads to a modification of the diffusion of the donor and/or acceptor so as to affect energy transfer may be used in accordance with the present invention. Thus, for example, in principle any suitable binding reaction may be used (e.g. an immune reaction (i.e. the specific binding reaction between an antibody and an antigen)).

Examples of selected species which therefore may be

detected in accordance with the present invention are antibodies, antigens, lectins, other bio-active molecules, cells and cell fragments.

Thus, the present invention may be used in immunoassay, immunosensing and other biosensing applications.

The interaction which gives rise to a modification in diffusion may, for example, take place in free solution (i.e. three dimensional diffusion), in a film (i.e. two dimensional diffusion), or between a solution and a suitable surface (e.g. such a surface as may be provided by latex particles or micro-titre plates). For example, the donor and/or acceptor may be linked to a suitable surface (e.g. that provided by latex particles (e.g. of 100 nm particle size) or micro-titre plates).

By way of example, the donor and acceptor may be freely diffusing in solution and activation energy applied; because the free diffusing enhances the transfer of energy between the donor and acceptor, the donor energy will be observed to be relatively low and the acceptor energy will be observed to be relatively high. On introduction of the selected species, interaction thereof with the donor and/or the acceptor leads to a reduction in the diffusion rate of the donor and/or acceptor which gives rise to a reduction in energy transfer from donor to acceptor; thus, the energy of the donor will be observed to increase and that of the acceptor to decrease.

The interaction may be, for example, a binding reaction between an antibody and an antigen; thus initiating an immune reaction may lead to a modification of diffusion in accordance with the present invention.

The donor and/or acceptor, by way of example, may be linked to proteins and used in free solution.

Thus, for example, a donor comprising a chelated complex containing a lanthanide ion may be conjugated or covalently linked to a protein. An acceptor may be conjugated or covalently linked to a protein.

Examples of proteins suitable for linking to a donor and/or an acceptor are immunoglobulins and lectins of specific binding characteristics and carrier proteins such as bovine serum albumin and poly-l-lysine.

By way of example, in applications of the invention where it is likely that interfering fluorescence may occur (e.g. in immunoassay or immunosensing where non-specific protein fluorescence from biological material in body fluids, such as serum or urine, may occur) time resolved fluorescence or phosphorescence measurements may be used thereby to eliminate or substantially avoid interfering fluorescence; by way of example, time resolved fluorescence or phosphorescence measurements may be facilitated by the use of a donor capable of exhibiting a relatively long excited state lifetime (e.g. 10^{-3} secs).

The majority of fluorescence processes occur over a time period of 10^{-9} to 10^{-7} seconds; this includes non-specific protein fluorescence. Difficulties arising from non-specific fluorescence may be overcome, or substantially avoided, in accordance with the present invention. Thus, for example, a sample containing a donor and acceptor in accordance with the present invention may be subjected to excitation (e.g. by means of a xenon flash lamp) and then excitation ceased; after a suitable delay to allow the decay of non-specific, and therefore unwanted fluorescence, a suitable monitoring system may be activated to measure the remaining phosphorescence signals.

Lanthanide ions can exhibit relatively long excited

lifetimes (of the order of 10^{-3} seconds) and may be used in accordance with the present invention. The fluorescence of inorganic salts of the lanthanides is, however, normally weak due to the energy absorption of the ion itself being low. However, the fluorescence may be significantly enhanced by an energy transfer process if a lanthanide ion is complexed to a suitable organic species; this may be termed ion fluorescence and is notably displayed by chelated ions of Sm, Eu, Tb and Dy. Examples of complexing agents that may be used in accordance with the present invention are dipicolinic acid, quinolene-2, 3-dicarboxylic acid, quinaldic acid, 2, 3-napthalene dicarboxylic acid, CDTA (trans-1, 2-diaminocyclohexane-N, N, N', N', tetracetic acid), quin 2 (2-[[2-bis(carboxymethyl)-amino-5-methylphenoxy]-methyl] -6-methoxy-8-bis(carboxymethyl)-amino quinoline), diethylene-triaminepentacetic acid, aurin tricarboxylic acid, and the conjugate of p-amino salicylic acid with diethylene-triamine pentacetic acid anhydride.

Where a donor is activated by an energy transfer process from an organic complexing agent it is preferred that the acceptor has appropriate properties to permit energy transfer.

Thus, for example the acceptor may have a satisfactory absorbance at the emission wavelength of the donor (i.e. the donor and acceptor energy levels should be preferably appropriately matched. Also, for example, the acceptor may readily emit transferred energy as observable fluorescence or phosphorescence.

Examples of acceptors (which, if desired, may be attached to suitable proteins) which may be used in accordance with the present invention are Rhodamine B, Texas red (Sulphorhodamine 101 acid chloride), carbazine 720 and

thionine. By way of example, in principle, any suitable dye capable of appropriate observable energy emission (e.g.fluorescence energy or phosphorescence energy) may be used.

As an alternative, or in addition, to substantially avoiding problems of non-specific fluorescence in biological samples as hereinbefore disclosed, problems of non-specific fluorescence may also be reduced or substantially avoided by choosing the donor appropriately; thus, for example, where a lanthanide ion is to act as a donor in combination with a suitable complexing agent, the complexing agent (e.g. a chelating agent) for the lanthanide ion may be chosen to have an absorption wavelength out of the region of absorption wavelengths of possible interfering proteins.

It is to be understood that the donor and/or acceptor should not be adversely affected to any unacceptable degree by the materials with which they are likely to be contacted in use. Thus, for example, a donor and/or an acceptor for use in a biological system should be suitably stable in contact with the materials in the biological system to enable the process of the invention to be carried out satisfactorily.

It is further to be understood that the present invention may be applied in, for example, homogeneous and non-homogeneous systems. The homogeneous system offers the possibility of a simple one-step procedure (e.g. one-step immunoassay).

In one embodiment of the present invention a donor may be linked to a suitable surface, an acceptor, including a first antibody or a first antigen, is allowed to diffuse freely thereto, a second antigen or a second antibody is provided, a species to be detected is introduced, said species having site for undergoing a specific binding

reaction with the said first antibody or antigen and a site for undergoing a specific binding reaction with the said second antibody or antigen, and the change in energy signal from the donor and/or acceptor is monitored whereby the presence of the selected species is detected by means of observing changes in the energy levels of the donor and/or acceptor brought about by a reduction in the diffusion of the donor and/or acceptor caused by the binding reactions.

It will be appreciated that whether the donor or acceptor contains an antibody or antigen will depend upon whether the species to be detected comprises respectively an antigen or an antibody. It will be understood that the immediately preceeding embodiment represents a "sandwich" form of immunoassay.

The surface of the immediately preceeding embodiment may comprise, by way of example, the surface of a latex particle or the surface of a titre plate.

In the immediately preceeding embodiment, if desired, the second antibody or antigen may be attached to a suitable surface (e.g. the surface of a latex particle or the surface of a titre plate).

By way of example, a donor comprising chelated terbium may be attached to latex particles and allowed to diffuse freely in solution, an acceptor comprising Rhodamine linked to a first antibody for HCG (human chorionic gonadotrophin) is added, a second antibody for HCG attached to latex particles is introduced, the solution subjected to excitation, and the energy levels of the donor and/or acceptor are monitored, binding reactions are allowed to proceed between the first and second antibodies and the HCG and the change in energy levels caused by the effect of the binding reactions on diffusion are monitored thereby to

indicate the presence of HCG.

The present invention may also be employed in carrying out detection of species by displacement assay (e.g. displacement immunoassay).

It is to be understood that the invention may be applied to competitive assay and non-competitive immunoassay.

Excitation energy may be applied in any suitable manner for example by means of a xenon flash lamp (e.g. at a wavelength of 303 nm). The wavelength of the excitation maybe chosen as is appropriate for a given application and may be chosen on the basis of, for example, the absorption or emission wavelength of the donor and/or of the acceptor. Typical wavelengths may be in the range 200 nm - 700 nm.

Fluorescence and/or phosphorescence monitoring may be effected by use of any suitable apparatus an example of which is a Perkin Elmer LS-5 Luminescence Spectrometer.

By way of example it may be noted that in certain applications it may be advantageous to choose an acceptor (e.g. a dye which can be attached to a protein) which can accept energy from a donor (e.g. a terbium ion associated with a chelating agent) and which also has a sufficiently large Stokes shift to emit light at a wavelength beyond that of the emission wavelength of the donor.

According to a further aspect the invention provides apparatus for effecting detection of a selected species by a process in accordance with the present invention, comprising means for bringing the selected species into communication with a donor and an acceptor, means for providing excitation energy and means for monitoring an energy signal from the donor and/or acceptor whereby the presence of the selected species may be detected.

In one embodiment, an apparatus in accordance with the present invention, and suitable for a "sandwich" immunoassay, may include a capillary - fill cell having coated on its opposite internal walls a layer of a soluble gel (e.g. an hydrogel) containing a donor and/or acceptor and a layer containing an immobilised antibody or antigen.

In operation the cell is filled with liquid containing selected species to be detected whereupon donor and acceptor are released from the gel and the selected species reacts with the immobilised antibody or antigen and with the acceptor.

By applying excitation energy to the cell and monitoring the emission of energy (either backscattered energy or forward emitted energy) the presence of the selected species may be detected by comparison with energy emissions obtained when the cell is filled with a reference liquid containing none of the selected species.

By way of further example, an apparatus in accordance with the present invention may include a device which has a plate of transparent material carrying a porous layer, which porous layer contains a donor, an acceptor and an antibody or an antigen.

The porous layer may be, for example, a transparent ceramic, glass or plastic filter material.

In operation the device may be exposed to a solution containing a selected species to be detected whereupon the selected species interacts with the acceptor and antibody or antigen; excitation energy may be applied and energy emission by the donor and/or acceptor monitored whereby presence of the selected species may be detected by comparison with energy emission obtained when the device

is contacted with a reference solution containing none of the selected species.

It is to be understood that because diffusion rates can be affected by such factors as viscosity and temperature it may be advantageous to optimise such factors for a given application.

It is also to be understood that where a relatively slowly diffusing acceptor is to be used, for example where an acceptor is attached to relatively large molecule (e.g. a protein such as an immunoglobulin), the use of a donor having a relatively long excited lifetime may be utilised to facilitate the exchange of energy from the donor to the acceptor.

As a further alternative, given by way of further example, the monitoring of the effect of a modification of diffusion of a donor and/or an acceptor upon energy transfer from the donor to the acceptor may be carried out by monitoring a change in lifetime of an emitting donor and/or acceptor.

The monitoring of a change in lifetime may be effected, for example, by measuring intensities of emission of an emitting donor and/or acceptor over two or more time intervals.

Thus, for example, the ratio of intensities over the time intervals may be used to monitor the lifetime characteristics of the donor and/or acceptor.

The use of change in lifetime characteristics enables difficulties presented by the presence of species giving rise to interfering absorption, scattering or fluorescence to be substantially overcome or avoided.

It will be understood that, as hereinbefore disclosed, interfering species may give rise to, for example, adsorption and/or scattering of excitation energy when using emission intensity measurements to monitor the transfer of energy from a donor to an acceptor.

However, although intensity measurements as such may be affected by the presence of such interfering species, the emission lifetime characteristics of a donor and/or an acceptor will be affected substantially only by the presence or absence of the selected species and not by the presence of such interfering species.

For example, the lifetime characteristics of a donor and/or acceptor may be monitored by a method which utilises (by means of integration) areas under a curve obtained by plotting emission intensity against time.

Thus, for example, excitation energy may be applied (e.g. by means of a xenon flash lamp) to a sample containing a selected species to be detected and other species which can give rise to interfering non-specific fluorescence, and the intensity of emission from the sample is plotted against time.

Following application of the excitation energy there will be a time period (t_1) in which a peak of emission will be observed as interfering species give rise to non-specific fluorescence. After this period t_1 the intensity of emission from the donor or acceptor is monitored as it decays over, say, two further periods of time (t_2 and t_3).

The energy emissions for period t_1 are disregarded and the intensity/time plot is integrated separately for periods t_2 and t_3 to give integrated values I_2 and I_3 respectively.

The ratio of I_2 and I_3 enables the lifetime emission characteristics of the donor or acceptor to be used to monitor the effect of a modification of diffusion of a donor and/or acceptor upon energy transfer from the donor to the acceptor.

By way of example, the following two systems may be considered:

Thus, in a first system a donor absorbs much energy, little energy is transferred to the acceptor and the acceptor emits little energy.

In a second system a donor absorbs little energy, efficient energy transfer occurs and the acceptor emits little energy.

A simple intensity measurement does not offer an effective means of differentiality between the first and second systems because the net observation is that of little energy emission from the acceptor.

However, by use of lifetime changes in emissions, obtained by making intensity measurements over different periods of time as hereinbefore described, the first and second sytems may be differeniated.

The present invention will now be further described, by way of example only, as follows:

Example

DPAS (1mg) dissolved in distilled water (1 ml) was added to

$TbCl_3$ (1 mg) dissolved in distilled water (1 ml) and left to react for 10 minutes at room temperature.

[DPAS is formed by reacting DTPA (diethylenetriaminepentacetic acid anhydride) with para-aminosalicylic acid].

To the resulting solution (2 ml) amino-silica beads (20 mg; ex Waters) were added together with a solution of 1-ethyl-3(-3 dimethylaminopropyl) carbodiinide(1 mg in distilled water (1 ml)) to give a suspension containing beads which will hereinafter be referred to as "Tb-DPAS-SiO_2 beads".

Intensity measurements were made using a xenon flash lamp and a Perkin Elmer LS-5 Luminescence Spectrometer as follows:

Stage 1. A sample of the suspersion containing the Tb-DPAS-SiO_2 beads (20 ul) was added to 100% rabbit serum (2 mls; Globepharm Ltd).

This resulting mixture was subjected to the application of excitation energy by means of the xenon flash lamp and emission intensities were measured (in arbitary units) using a first time gate of 0.1 to 1.5 mS and a second time gate of 1.6 to 5.0 mS.

It will be understood that under the conditions of Stage 1 no acceptor species is present and therefore no diffusion enhanced energy transfer can take place.

Stage 2. To the mixture of the suspersion containing Tb-DPAS-SiO_2 beads and rabbit serum, a solution of Texas Red conjugated antimouse IgG was added (20 ul of 1 mg/1 ml solution; Jackson Immunochemicals) and the resulting

mixture was subjected to excitation energy and intensity measurements in accordance with the procedure described in Stag 1. It will be appreciated that Texas Red conjugated antimouse IgG is an acceptor and hence diffusion enhanced energy transfer can place under the conditions of Stage 2.

Stage 3. To the mixture formed in Stage 2 a mouse IgG-agarose beads suspersion was added (50 ul; (antibody concentration 0.5 mg/ml); Sigma) and the resulting mixture was subjected to excitation energy and intensity measurements were made using the procedure described in Stage 1. In the case of Stage 3, however, first and second gated emission intensities were measured both 15 minutes and 45 minutes after addition of the mouse IgG-agarose bead suspersion.

It will be appreciated that mouse IgG is the selected species to be detected in the present Example and that the presence of the mouse IgG, present in the form of the mouse IgG-agarose beads, is detected by virtue of the fact that the mouse IgG-agarose beads bind to the Texas Red-antimouse IgG such that there is a significant reduction in diffusion enhanced energy transfer.

Stage 4. An excess of "free" mouse IgG (100 ul of mouse IgG solution; (antibody concentration 0.5 mg/ml); Sigma) was added to the mixture formed in Stage 3.

Excitation energy was applied to the resulting mixture by the procedure of Stage 1 and first and second gated emission intensities from the mixture were made by the procedure of Stage 1.

It will be appreciated that the excess "free" mouse IgG displaces the mouse IgG-agarose beads from the Texas Red-antimouse IgG/mouse IgG-agarose bead species formed in

Stage 3 thereby to give a Texas Red-antimouse IgG species.

It will be appreciated further that the latter species, being much smaller than the former species, diffuses faster and hence diffusion enhanced energy transfer can once more take place.

The first time gate of 0.1 - 1.5 mS was chosen substantially to avoid prompt fluorescence which occurs, primarily from serum proteins, is a time of less than 0.1 mS.

The integrated intensities over the gating times of 0.1 - 1.5 mS and 1.6 - 5.0 mS for Stages 1 to 4 and the ratios of the integrated intensities for the first and second time gates are given in the following Table:

Table

Stage No.	Integrated Intensity		
	Gate 1	Gate 2	Ratio $\frac{\text{Gate 1}}{\text{Gate 2}}$
Stage 1	8.16	3.00	2.72
Stage 2	8.41	2.90	2.90
Stage 3 (after 15 mins)	7.24	2.55	2.84
Stage 3 (after 45 mins)	8.65	3.20	2.70
Stage 4	7.86	2.72	2.89

On comparing the results given in the Table for Stages 2 and 3 (after 45 mins) it can be seen that there is a significant reduction in the integrated intensity ratios resulting from

the addition of species to be determined which causes a significant reduction in diffusion enhanced energy transfer.

It will be appreciated that a calibration curve may be prepared by use of standard preparations containing known concentrations of species to be detected and the calibration curve thus prepared may subsequently be used in determining concentrations of selected species in test samples.

It will be noted that 15 minutes after adding mouse IgG-agerose beads (Stage 3) the observed integrated intensity is relatively small (7.24 for gate 1). This is, however, caused predominantly by light scattering from beads and not an increase in diffusion enhanced energy transfer.

Thus, in this Example, intensity measurements alone are not the best indicators of the degree of diffusion enhanced energy transfer. However, the use of the ratio of gated limetime intensities does act as an indicator of the degree of diffusion enhanced energy transfer.

Furthermore, on comparing the ratios obtained in Stage 2 and 4, it will be seen that they are similar as would be expected when diffusion enhanced energy transfer is not affected by the presence of species to be detected.

However, in the particular Example, the intensity drops from 8.41 to 7.86 due, in part, to further dilution.

This demonstrates that observed integrated intensity is concentration dependant whereas the ratio of integrated intensities is not.

Thus the use of integrated intensity ratios may be used to avoid the need for accurately known dilutions.

Claims

1 A method for detecting a selected species which comprises monitoring the effect of a modification of diffusion of a donor and/or of an acceptor upon energy transfer from the donor to the acceptor, the modification of diffusion resulting from interaction with the selected species.

2 A method as claimed in Claim 1 wherein the diffusion coefficient of an energy donor and/or of an energy acceptor is modified by an interaction involving the selected species whereby diffusion enhanced energy transfer from a donor to an acceptor is affected.

3 A method as claimed in Claim 2 wherein the selected species is arranged to interact with the donor and/or with the acceptor so as to reduce transitional diffusion of the donor and/or of the acceptor during the lifetime of the donor in an excited state whereby the rate of energy transfer from donor to acceptor is reduced.

4 A method as claimed in any one of the preceding Claims wherein excitation energy is applied and energy emission of the donor and/or acceptor is monitored.

5 A method as claimed in any one of the preceding Claims wherein energy emission of the donor and/or acceptor is observed as fluorescence energy or as phosphorescence energy.

6 A method as claimed in any one of the preceding Claims wherein monitoring the effect of a modification of diffusion of a donor and/or an acceptor upon energy transfer from the donor to the acceptor is carried out by monitoring a change in lifetime of an emitting donor and/or acceptor.

7 A method as claimed in any one of the preceding Claims wherein the interaction which gives rise to a modification in diffusion takes place in free solution.

8 A method as claimed in any one of Claims 1 to 6 wherein the interaction which gives rise to a modification in diffusion takes place in a film.

9 A method as claimed in any one of Claims 1 to 6 wherein the interaction which gives rise to a modification in diffusion takes place between a solution and a surface.

10 A method as claimed in Claim 9 wherein the surface is provided by latex particles or by a mirco-titre plate.

11 A method as claimed in Claim 9 or Claim 10 wherein a donor is linked to a suitable surface, an acceptor, including a first antibody or a first antigen, is allowed to diffuse freely thereto, a second antigen or a second antibody is provided, a species to be detected is introduced, said species having a site for undergoing a specific binding reaction with the said first antibody or antigen and a site for undergoing a specific binding reaction with the said second antibody or antigen, and change in energy signal from the donor and/or acceptor is monitored whereby the presence of the selected species is detected by means of observing changes in the energy levels of the donor and/or acceptor brought about by a reduction in the diffusion of the donor and/or acceptor caused by the binding reactions.

12 A method as claimed in any one of the preceding Claims wherein the donor and/or acceptor is/are linked to a protein.

13 A method as claimed in Claim 12 wherein a donor

comprising a chelated complex containing a lanthanide ion is conjugated or covalently linked to a protein.

14 A method as claimed in Claim 12 wherein an acceptor is conjugated or covalently linked to a protein.

15 A method as claimed in any one of Claims 12 to 14 wherein the protein is an immunoglobulin or a lectin of specific binding characteristics.

16 A method as claimed in any one of Claims 12 to 14 wherein the protein is a carrier protein comprising bovine serum albumin or poly-l-lysine.

17 A method as claimed in any one of the preceding Claims wherein a donor includes a lanthanide ion comprising Sm, Eu, Tb or Dy.

18 A method as claimed in any one of the preceding Claims wherein a donor includes a complexing agent comprising dipicolinic acid, quinolene-2, 3-dicarboxylic acid, quinaldic acid, 2, 3-napthalene dicarboxylic acid, CDTA (trans-1, 2-diaminocyclohexane-N, N, N', N', tetracitic acid), quin 2 (2-[[2-bis(carboxymethyl)-amino-5-methylphenoxy]-methyl] 6-methoxy-8-bis(carboxymethyl)-amino quinoline), diethylene -triaminepentacetic acid, aurin tricarboxylic acid, or the conjugate of p-amino salicylic acid with diethylene-triamine pentacetic acid anhydride.

19 A method as claimed in any one of the preceding Claims wherein an acceptor comprises Rhodmine B, Texas Red (Sulphorhodamine 101 acid chloride), carbazine 720 or thionine.

20 A method as claimed in any one of the preceding Claims wherein the interaction includes a binding reaction between an antibody and an antigen such that immunoassay or immunosensing is effected.

21 A method as claimed in any one of the preceding Claims wherein the selected species to be detected is an antibody, an antigen, a lectin, a bio-active molecule, a cell or a cell fragment.

22 Apparatus for effecting detection of a selected species by a method as claimed in any one of the preceding Claims comprising means for bringing the selected species into communication with a donor and an acceptor, means for providing excitation energy and means for monitoring an energy signal from the donor and/or acceptor whereby the presence of the selected species may be detected.

23 Apparatus as claimed in Claim 22 which includes a capillary - fill cell having coated on its opposite internal walls a layer of a soluble gel containing a donor and/or acceptor and a layer containing an immobilised antibody or antigen.

24 Apparatus as claimed in Claim 22 or 23 which includes a device which has a plate of transparent material carrying a porous layer, which porous layer contains a donor, an acceptor and an aqntibody or an antigen.

25 Apparatus as claimed in Claim 24 wherein the porous layer is a transparent ceramic, glass or plastic filter material.

26 A method for detecting a selected species substantially as hereinbefore described with reference to the Example.

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